*Excerpta*

ACTA PHYSIOLOGICA SCANDINAVICA
1952. VOL. 26. FASC. 1

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# Ascorbic Acid as a Test Substance for Measuring Relative Changes in the Rate of Plasma Flow through the Ciliary Processes

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R. UNIV. BIBLIOTHEEK LEIDEN

UPPSALA 1952

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# Ascorbic Acid as a Test Substance for Measuring Relative Changes in the Rate of Plasma Flow through the Ciliary Processes

BY

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AN ACADEMIC THESIS

which, by Due Permission of the Most Experienced Faculty of Medicine of the University of Uppsala, will be Publicly Defended on Saturday April 26th 1952 at 10 a. m. in the Lecture Room of the Pharmacological Institute, for the Degree of Doctor of Medicine

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**Acta Physiologica Scandinavica 1952. Vol. 26. Fasc. 1.**

From the Pharmacological Institute of the University of Uppsala.

# Ascorbic Acid as a Test Substance for Measuring Relative Changes in the Rate of Plasma Flow through the Ciliary Processes.

## I. The Effect of Unilateral Ligation of the Common Carotid Artery in Rabbits on the Ascorbic Acid Content of the Aqueous Humour at Varying Plasma Levels.

By

ERIK LINNÉR.

Received 18 February 1952.

Soon after the discovery of ascorbic acid its concentration in the aqueous humour and in the lens was found to be appreciably higher than in the plasma, in man and in many kinds of animals (MÜLLER 1932, 1933, HARRIS 1933).

Different theories have been advanced to explain the high concentration of ascorbic acid in the aqueous humour. MÜLLER, BUSCHKE, GUREWITSCH, and BRÜHL, (1934), using cattle and rabbits, and FISCHER (1934), using cattle, suggested that the ascorbic acid in the aqueous humour was synthesized by the lens. BAKKER (1936), using rabbit lenses, was not, however, able to show any synthesis of ascorbic acid.

It has further been suggested that a transfer of ascorbic acid takes place from the plasma to the aqueous humour. VAN ECKELEN, EMMERIE, JOSEPHY, and WOLFF, (1934), and CIOTOLA (1936), postulated a transfer as ascorbic acid. CIOTOLA showed that intravenous injection of ascorbic acid greatly raised the concentration of ascorbic acid in its reduced form in the aqueous humour of aphakic eyes. On the other hand that dehydro-ascorbic acid was transferred to the aqueous humour, and reduced to ascorbic acid by the lens, was postulated by MÜLLER and BUSCHKE (1934), and that the permeability of the blood-aqueous barrier to ascorbic

acid in the direction from the aqueous humour to the blood was low was suggested by MONJUKOWA and FRADKIN (1935), and by GOLDMANN and BUSCHKE (1935 a).

FRIEDENWALD, BUSCHKE, and MICHEL, (1943), suggested that the ascorbic acid of the plasma is oxidized to dehydro-ascorbic acid by the ciliary epithelium, and then transferred to the aqueous humour of the posterior chamber. A new investigation by KINSEY (1950) using rabbits speaks, however, in favour of a transfer of ascorbic acid itself from the plasma to the posterior chamber as ascorbic acid. From there it flows into the anterior chamber with some loss, since the lens utilizes ascorbic acid. Dehydro-ascorbic acid has not been found to be reduced to ascorbic acid by the intact lens. LANGHAM (1950), using cats and rabbits, raised the concentration of dehydro-ascorbic acid in the plasma to a high level; he found a high concentration of ascorbic acid in the aqueous humour but not, however, a large enough increase in the plasma ascorbic acid to explain the high values in the aqueous humour. The methods for estimating low concentrations of ascorbic acid in plasma are, however, so unsatisfactory (for review see GYÖRGY, 1950) that the figures cannot be accepted as definite proof of a transfer of dehydro-ascorbic acid from the plasma into the eye.

The same species of animals have not been used by all investigators. PODESTA and BAUCKE (1938) investigated the cause of the high concentration of ascorbic acid in the aqueous humour in different species of animals in order to see if there was any difference between them, and found cattle lenses to be capable of synthesizing ascorbic acid, but not rabbit lenses. Guinea-pig lenses synthesized moderate amounts, but only in the presence of an insufficient supply of ascorbic acid. The difference between various species may explain the inconsistent results.

In short, the evidence at present available for such animals as guinea-pigs supplied with enough ascorbic acid, and rabbits, speaks in favour of a transfer of ascorbic acid from the plasma to the aqueous humour. It is not quite clear if the dehydro-ascorbic acid of the plasma can also be a source of the ascorbic acid of the aqueous humour.

The possible importance of circulatory factors in connection with the ascorbic acid content of the aqueous humour was first pointed out by GOLDMANN and BUSCHKE (1935). These authors believed the concentration of ascorbic acid to be dependent on

the concentration of dehydro-ascorbic acid in the blood, and that the concentration of ascorbic acid in the aqueous humour roughly follows the blood concentration of dehydro-ascorbic acid. According to their view dehydro-ascorbic acid diffuses from the blood into the aqueous humour and is then reduced to ascorbic acid by the lens, the barrier from the aqueous humour to the blood being closed to ascorbic acid. They discussed the following factors which they thought might be of importance for the concentration of ascorbic acid in the aqueous humour:

1) the blood concentration of dehydro-ascorbic acid,
2) the state of the blood-aqueous barrier,
3) the reducing-capacity of the lens.

Under the heading 'the state of the blood-aqueous barrier' they considered the magnitude of the blood flow through the uveal capillaries, the permeability of the capillary wall to dehydro-ascorbic acid, and the permeability of the capillary wall to ascorbic acid.

Continuing the experiments on factors of importance in connection with the concentration of ascorbic acid in the aqueous humour, BUSCHKE (1936) investigated the rôle played by the blood flow through the uveal capillaries, and by the permeability of the blood-aqueous barrier. He showed that local application of adrenaline decreased, and that of acetylcholine increased, the concentration of ascorbic acid in the aqueous humour. Discussing his findings he seemed to accept only a mechanism of diffusion. The concentration of ascorbic acid in the aqueous humour was dependent on the concentration of dehydro-ascorbic acid in the blood, and on the amount of blood coming into contact with the aqueous humour. BUSCHKE refrained from a more detailed analysis of the mechanism but definitely connected the observed experimental changes in the concentration of ascorbic acid in the aqueous humour with the influence of adrenaline and acetylcholine on the uveal circulation.

If, as seems probable, the ascorbic acid of the aqueous humour comes from the plasma, the blood-supply might well be the limiting factor for the amount of ascorbic acid which can be transferred to the aqueous humour if the plasma-concentration of ascorbic acid (or dehydro-ascorbic acid) is low enough (LINNÉR 1951 a, 1951 b). This would have important consequences. It would mean that the clearance of ascorbic acid by the secretory part of the eye can be used to measure the rate of plasma flow

through the ciliary processes in the same way as the clearance of para-amino-hippuric acid, or Diodrast, is used in the case of the kidneys. The view is supported by previous results (Linnér 1951 a, 1951 b) where it was shown that unilateral carotid ligation in rabbits reduces the ascorbic acid concentration of the aqueous humour to 80 per cent of that in the control eye. This difference is obtained at physiological values of ascorbic acid.

## Plan of the Present Study.

This study was concerned with the changes in the concentration of ascorbic acid in the aqueous humour caused by changes in the rate of blood-flow through the eye, at plasma-concentrations varying over a wide range below the saturation level. The same principle was followed as in the preliminary studies (Linnér 1951 a, 1951 b). The blood flow through one eye was changed by means of unilateral carotid ligation, and the other eye was used as a control. The ratio between the concentration of ascorbic acid in the aqueous humour of the two eyes was used throughout. Since the effect of carotid ligation varies in individual animals a sufficiently large number of rabbits was used to permit a statistical levelling of the variations.

In another group of rabbits the plasma-concentration of ascorbic acid was raised to above the saturation level in order to analyse the maximal rate of transfer of ascorbic acid across the blood-aqueous barrier.

## Materials and Methods.

Male albino rabbits weighing 1.8—2.5 kg were used. They were kept on a diet of hay, oats, and water, *ad lib.*; some of the animals investigated in the spring were given green grass. One common carotid artery was ligated under a short-acting barbiturate (Numal 'Roche' 0.6 ml/kg body-weight) and 18—20 hours later the aqueous samples were collected from the two eyes, one immediately after the other. The eyes were proptosed after the application of a local anaesthetic (2 per cent 'Xylocaine' (Astra)). The aqueous humour from each eye was immediately divided between 3 small weighed tubes containing 5 per cent metaphosphoric acid. The amount of aqueous humour was determined by weight-difference, and ascorbic acid was then titrated with dichlorophenolindophenol. The titration was completed within 30 seconds of its start. An Agla syringe was used for the titration, and adequate mixing was assured by magnetic stirring. Blood was obtained by heart-

puncture with a heparinized syringe, and was immediately centrifuged. One volume of plasma was mixed with 2 volumes of 5 per cent metaphosphoric acid. After further centrifuging exactly 0.5 ml of the clear fluid containing ascorbic acid was titrated in the same way as the aqueous humour. Three determinations were made on each sample of aqueous and of blood; the arithmetic mean was used.

In order to get a high ascorbic acid level definitely exceeding the saturation level (cf. p. 67), the rabbits in one group were given an intravenous infusion over a period of 6 hours of a freshly made 3.3 per cent solution of ascorbic acid with pH adjusted to 5.5—6.5 by means of NaOH. The rate of infusion was 2 ml per hour.

Standard statistical methods were used according to the formulae given in BONNIER-TEDIN (1940). The standard error of a single determination was calculated by means of analysis of variance as 'variance within classes'. The regression line was calculated according to the method of least squares.

## Results.

Table 1 shows the standard error of a single determination.

**Table 1.**

*The standard error of a single determination calculated by means of analysis of variance, i. e. the technical error arising from titration, weighing, etc., of each of the three determinations on every aqueous- or plasma-sample.*

Three determinations have been made on each of 30 samples of aqueous humour from 15 different animals, and of plasma from 30 different animals.

| Ascorbic acid in mg% | | |
|---|---|---|
| Aqueous humour | Plasma | |
| | Low concentration | High concentration |
| m = 18.92<br>s = 0.46<br>n = 90 | m = 0.59<br>s = 0.04<br>n = 90 | m = 13.90<br>s = 0.38<br>n = 90 |

Symbols: m = arithmetic mean
s = standard error of a single determination
n = number of determinations

Table 2 shows a comparison of the ascorbic acid content of the aqueous humour of the two eyes under physiological conditions. The standard deviation of the ratio is of the same order of magnitude as the standard error of a single determination. Therefore it is justifiable to regard both eyes as equal as far as the ascorbic acid content is concerned, and to use one eye as

**Table 2.**

*A comparison between the ascorbic acid content of the aqueous humour of the two eyes under physiological conditions.*

The aqueous humour was removed from the two eyes, one immediately after the other. All concentrations in mg ascorbic acid per 100 ml.

| Ascorbic acid in mg% | | | Ratio: Right / Left |
|---|---|---|---|
| Aqueous humour | | Plasma | |
| Right eye | Left eye | | |
| m = 15.63<br>s = 3.07<br>e = ± 0.64<br>n = 23 | m = 15.57<br>s = 3.15<br>e = ± 0.66<br>n = 23 | m = 0.60<br>s = 0.06<br>e = ± 0.01<br>n = 23 | m = 1.00<br>s = 0.02<br>e = ± 0.004<br>n = 23 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

a control when unilateral experiments are made. The figures of Goldmann and Buschke (1936) show a similar result.

According to Schneider and Schneider (1934) ligation of one common carotid artery causes an increase in the rate of blood flow through the common carotid artery on the other side. In order to discover if the concentration of ascorbic acid of the aqueous humour was affected by this ligation the following experiments were performed. The aqueous humour was removed from one eye and the common carotid artery on the same side was then ligated. 18—20 hours later the aqueous humour was removed from the eye on the non-ligated side. Table 3 shows the results. The difference between the group of rabbits in which the carotid artery was ligated, and a control group operated upon in the same way but without ligation of the artery, is statistically not significant ($0.05 < P < 0.1$).

The difference between this control group (2) and a group of rabbits without operation (3) where the samples of aqueous humour were taken at the same interval is statistically not significant ($0.02 < P < 0.05$). But the difference between group (1) and (3) is statistically significant ($P < 0.001$). Therein might be included both the effect of ligation of one carotid artery on the blood flow on the opposite side, and other factors caused by the operation.

**Table 3.**

*The effect of ligation of the common carotid artery on the ascorbic acid content of the aqueous humour on the non-ligated side.*

1) The aqueous humour was removed from one eye (I); the carotid artery was then ligated on the same side. 18—20 hours later the aqueous humour was removed from the eye on the non-ligated side (II). 2) As a control exactly the same operation was performed but the carotid artery was not ligated. 3) A group of rabbits without operation where the samples of aqueous humour were taken at the same interval. All concentrations in mg ascorbic acid per 100 ml.

| | Ascorbic acid in mg% | | |
|---|---|---|---|
| | Aqueous humour | | Ratio: |
| | I | II | II/I |
| 1) Group with ligation of the carotid artery. | m = 21.51<br>s = 5.64<br>e = ± 1.37<br>n = 17 | m = 24.28<br>s = 5.93<br>e = ± 1.44<br>n = 17 | m = 1.14<br>s = 0.12<br>e = ± 0.03<br>n = 17 |
| 2) Control group: the same operation but the carotid artery not ligated. | m = 20.69<br>s = 4.39<br>e = 1.06<br>n = 17 | m = 21.81<br>s = 3.97<br>e = ± 0.96<br>n = 17 | m = 1.07<br>s = 0.13<br>e = ± 0.03<br>n = 17 |
| 3) Control group: no operation at all. | m = 23.38<br>s = 6.27<br>e = ± 1.57<br>n = 16 | m = 23.18<br>s = 5.98<br>e = ± 1.49<br>n = 16 | m = 1.00<br>s = 0.09<br>e = ± 0.02<br>n = 16 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

Table 4 shows the ratio between the ascorbic acid content in the aqueous humour of the eye influenced by carotid ligation, and of the control eye. In the whole series of 86 rabbits the ratio was $0.83 \pm 0.01$. The rabbits with a low ascorbic acid level (10—18 mg per cent in the control eye) had a corresponding ratio of $0.86 \pm 0.02$, and the rabbits with a spontaneously high ascorbic acid level (26—38 mg per cent in the control eye) had a ratio of $0.80 \pm 0.03$. The difference between them is not statistically significant ($0.1 < P < 0.2$).

Figure 1 shows a scatter diagram of the ascorbic acid concentration in the aqueous humour of the eye influenced by carotid ligation, and of the control eye. The equation of the regression line was calculated to:

$$Y = 1.52 + 0.75\,x.$$

There is a certain disagreement between the value of the mean ratio, 0.83, and the equation of the regression line. In calculating

**Table 4.**

*The effect of ligation of the common carotid artery on the ascorbic acid content of the aqueous humour, at ascorbic acid levels below the saturation level.*

The carotid artery was ligated 18—20 hours before removal of the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

| | Ascorbic acid in mg% | | | |
|---|---|---|---|---|
| | Aqueous humour | | Plasma | Ratio: Ligated / Non-ligated |
| | Non-ligated side | Ligated side | | |
| The whole series of 86 rabbits. | m = 22.13<br>s = 5.92<br>e = ± 0.64<br>n = 86 | m = 18.18<br>s = 5.17<br>e = ± 0.56<br>n = 86 | m = 1.06<br>s = 0.41<br>e = ± 0.05<br>n = 71 | m = 0.83<br>s = 0.11<br>e = ± 0.01<br>n = 86 |
| Group of rabbits with a low ascorbic acid level (10—18 mg% in the control eye.) | m = 15.68<br>s = 2.22<br>e = ± 0.47<br>n = 22 | m = 13.40<br>s = 2.24<br>e = ± 0.48<br>n = 22 | m = 0.78<br>s = 0.37<br>e = ± 0.10<br>n = 15 | m = 0.86<br>s = 0.09<br>e = ± 0.02<br>n = 22 |
| Group of rabbits with a high ascorbic acid level (26—38 mg% in the control eye). | m = 30.92<br>s = 3.29<br>e = ± 0.76<br>n = 19 | m = 24.68<br>s = 4.80<br>e = ± 1.10<br>n = 19 | m = 1.25<br>s = 0.24<br>e = ± 0.06<br>n = 19 | m = 0.80<br>s = 0.13<br>e = ± 0.03<br>n = 19 |
| Group of rabbits with ligation of the right carotid artery. | m = 22.99<br>s = 6.09<br>e = ± 0.72<br>n = 71 | m = 18.87<br>s = 5.46<br>e = ± 0.65<br>n = 71 | m = 1.06<br>s = 0.41<br>e = ± 0.05<br>n = 71 | m = 0.83<br>s = 0.11<br>e = ± 0.01<br>n = 71 |
| Group of rabbits with ligation of the left carotid artery. | m = 18.08<br>s = 2.36<br>e = ± 0.61<br>n = 15 | m = 14.90<br>s = 2.57<br>e = ± 0.66<br>n = 15 | | m = 0.82<br>s = 0.07<br>e = ± 0.02<br>n = 15 |
| Group of rabbits with the stems of the kidney tied, and given PACA. | m = 26.27<br>s = 5.65<br>e = ± 0.93<br>n = 37 | m = 21.87<br>s = 5.06<br>e = ± 0.83<br>n = 37 | m = 1.16<br>s = 0.28<br>e = ± 0.05<br>n = 37 | m = 0.84<br>s = 0.12<br>e = ± 0.02<br>n = 37 |
| Group of rabbits given ACTH. | m = 17.68<br>s = 2.91<br>e = ± 0.71<br>n = 17 | m = 14.94<br>s = 2.94<br>e = ± 0.71<br>n = 17 | m = 0.58<br>s = 0.13<br>e = ± 0.03<br>n = 17 | m = 0.84<br>s = 0.08<br>e = ± 0.02<br>n = 17 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

the mean ratio all the single values have the same importance, but by calculating the equation of the regression line the values at the extremes of the variation range are given a greater im-

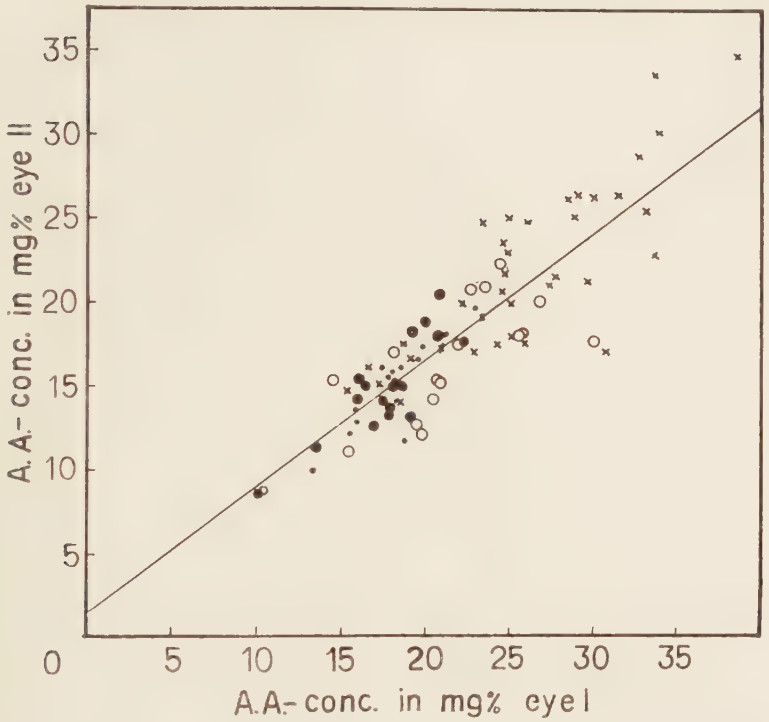


Fig. 1. The effect of ligation of the common carotid artery on the ascorbic acid content of the aqueous humour, at ascorbic acid levels below the saturation level. The carotid artery was ligated 18—20 hours before removal of the aqueous humour. Eye I: control eye. Eye II: eye influenced by carotid ligation. The concentrations in mg ascorbic acid per 100 ml. *Symbols:* Open circles: rabbits with ligation of the right carotid artery. Crosses: rabbits with ligation of the right carotid artery, with the stems of the kidney tied, and given PACA. Big black dots: rabbits with ligation of the right carotid artery, and given ACTH. Small black dots: rabbits with ligation of the left carotid artery.

portance. A more far-reaching statistical analysis has been avoided. It seems, however, justifiable to say that the scatter diagram shows no obvious tendency to be better represented by any but a straight regression line, and the ratio shows no tendency to vary at different ascorbic acid levels below saturation-level.

The series of 86 rabbits presented above is not quite homogeneous, but the differences are of such a nature that there is no reason to suppose that they have any influence on the ratio between the ascorbic acid concentration of the two eyes. In one

group of rabbits the carotid artery was ligated on the right, and in another group on the left, side. The ratio between the two eyes is 0.83 in the group which is ligated on the right side, and 0.82 in the group ligated on the left side. In one group of rabbits the distribution of p-acetylaminohippuric acid (PACA) between plasma and aqueous humour was determined at the same time as the ascorbic acid. In order to keep the plasma-concentration of PACA constant for several hours the stems of the kidneys were tied and the rabbits given PACA 18—20 hours before the puncture of the eye. The ratio between the two eyes is 0.84 compared with 0.83 in the whole group. In this investigation it was important to get as wide a variation as possible in the ascorbic acid level. Because the rabbits can synthesize ascorbic acid it is difficult to lower the level as much as is desired. FORTIER and SKELTON (1949) found that rats responded to stress with a triphasic variation of the plasma ascorbic acid. BÁRÁNY, BECKER and LINNÉR (1952) found an increased ascorbic acid level in the aqueous humour in rabbits two hours after administration of ACTH. Conversely in order to obtain a decrease in the ascorbic acid level, one group of rabbits was given ACTH, corresponding to 2 mg Armour's Standard LA 1 A in 0.1 ml volume, 12 hours before the puncture of the eyes. The ascorbic acid level was found to be low. These experiments, however, are not comprehensive enough to show decisively that the low level was caused by ACTH. The ratio between the two eyes is 0.84 compared with 0.83 in the whole group.

The effect observed for ascorbic acid is not an unspecific one. Carotid ligation has no influence on the total osmotic pressure of the aqueous humour (BÁRÁNY 1947 b), nor on the concentration of sodium (BÁRÁNY 1947 a). Data are also available for another acid, para-amino-hippuric acid (PAHA), which is transferred across the blood-aqueous barrier at a slow rate. This probably means that the rate of blood flow is not the limiting factor for the rate of transfer of PAHA through the blood-aqueous barrier. 32 rabbits with one carotid artery ligated were given an intravenous infusion of PAHA over a period of 6 hours. The ratio between the two eyes, calculated as the median was 1.02 (BÁRÁNY 1952). The deviation of a single value was however so great that a small difference between the two eyes could not be detected by means of this method. At a decrease in the rate of flow of aqueous humour the concentration of PAHA in the aqueous

**Table 5.**

*The effect of ligation of the common carotid artery on the ascorbic acid content of the aqueous humour at ascorbic acid levels above saturation level.*

The carotid artery was ligated 18—20 hours before removal of the aqueous humour. The saturation level was exceeded by means of intravenous infusion of an ascorbic acid solution over a period of 6 hours; plasma I was removed after 3 hours and plasma II at the same time as the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

| Ascorbic acid in mg% | | | | |
|---|---|---|---|---|
| Aqueous humour | | Plasma I | Plasma II | Ratio: Ligated / Non-ligated |
| Non-ligated side | Ligated side | | | |
| m = 50.64 | m = 51.19 | m = 11.89 | m = 13.94 | m = 1.01 |
| s = 5.64 | s = 5.66 | s = 4.97 | s = 4.01 | s = 0.04 |
| e = ± 1.29 | e = ± 1.30 | e = ± 1.28 | e = ± 0.92 | e = ± 0.008 |
| n = 19 | n = 19 | n = 15 | n = 19 | n = 19 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

humour would increase if the transfer of PAHA across the blood-aqueous barrier were not decreased to the same extent as the rate of flow of aqueous humour.

Above saturation level the supply exceeds the maximal rate of transfer of ascorbic acid across the blood-aqueous barrier. As shown by KINSEY (1947), and LANGHAM (1949), the saturation of the secretory part of the eye is reached at a plasma ascorbic acid level of about 3 mg per cent. In the experiments described here the saturation level was exceeded for 6 hours, *i. e.* long enough to yield steady state conditions in the eye. As shown in table 5 there is no difference between the ascorbic acid contents in the eye influenced by carotid ligation and in the control eye. At a rate of flow of aqueous humour of 2.75 cu.mm per minute (KINSEY and BÁRÁNY 1949) the maximal rate of transfer of ascorbic acid across the blood-aqueous barrier will be 1—1.5 microgram per minute.

A discussion will follow later when the results of further investigations concerning the same problem are published.

**Summary.**

In male rabbits the influence of unilateral carotid ligation on the concentration of ascorbic acid in the aqueous humour was investigated at varying plasma-levels of ascorbic acid. Over a wide range below saturation level the ratio between the concentrations in the aqueous humour on the ligated and the non-ligated sides was constantly $0.83 \pm 0.01$. Above saturation level there was no difference between the two eyes.

This investigation was supported by grants from the Research Fund of the Faculty of Medicine in the University of Uppsala, and from the Harald and Greta Jeansson Foundation. The technical assistance of Miss K. Nordström and Mr. A. Ojamaa is gratefully acknowledged.

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*EXCERPTUM*

**Acta Physiologica Scandinavica 1952. Vol. 26. Fasc. 1.**

From the Pharmacological Institute of the University of Uppsala.

# Ascorbic Acid as a Test Substance for Measuring Relative Changes in the Rate of Plasma Flow through the Ciliary Processes.

## II. The Effect of Unilateral Ligation of the Common Carotid Artery on the Blood Flow through the Uveal Tract as Measured Directly in a Vortex Vein.

By

ERIK LINNÉR.

Received 20 December 1951.

The circulation of the eye in lower mammals, such as rabbits, differs in certain respects from that of man (Koster 1895, Leber 1903). The eye receives its blood partly from the internal, and partly from the external, carotid arteries. The retina has its own vascular system. The uveal tract and sclera are supplied by the uveal circulation, and their venous drainage takes three different courses: (1) The main part is drained through the vortex veins. (2) A small part only of the sclera round the optic nerve is drained through certain small veins, the short posterior ciliary veins. (3) At the anterior border of the choroid lies the circle of Hovius, which is partly drained forwards through the anterior ciliary veins. In addition there are anastomoses going backwards from the circle of Hovius to the vortex veins, and these may largely replace the drainage through the anterior ciliary veins.

Because the venous outflow from the eye goes through several systems of veins, it follows that the total blood flow through the eye cannot be measured directly. However, most of the blood coming from the uveal tract passes through the four vortex veins.

This blood-flow has been the object of previous measurements. In all cases one of the vortex veins was exposed and cut, and the

blood running out of the eye collected over a certain period of time, during which a centrifugal backflow from the central part of the vein was prevented. The amount of collected blood multiplied by four gives an approximate value of the total blood flow through the uveal tract.

KANEKO (1925) used dogs under morphine anaesthesia. The blood was collected on filter-paper. The arithmetic mean obtained in four experiments was 0.00157 g/minute from one single vortex vein.

FISCHER, MEESMANN, and SONDERMANN, used rabbits. FISCHER (1930, 1931) collected the blood on filter-paper, the time in 6 of the 7 experiments being only 3 -6 seconds. He obtained an arithmetic mean of 0.326 g/minute from one single vortex vein.

MEESMANN (1930, 1932, 1951), on the other hand, collected the blood with a small cannula: but otherwise he used the same technique. He stated his results in cc/g eyeball-weight/minute. The arithmetic mean of 20 experiments reported in 1930 was 0.476 cc, and that of 5 experiments reported in 1932, 0.603 cc. To make possible a comparison with other results, this value must be recalculated. Unfortunately, MEESMANN does not indicate either the weight of the eyeball or the specific weight of the blood. When recalculating MEESMANN's result, the present author has therefore been forced to use his own values, taking the weight of the eyeball as 2.5 g, and the specific weight of the blood as 1.05. MEESMANN's arithmetic mean corresponds to 0.312 g/minute, and in the later group 0.396 g/minute, from one single vortex vein.

SONDERMANN (1932) collected the blood on filter-paper. From 21 experiments on 13 rabbits he obtained an arithmetic mean of 0.28 g/minute from one single vortex vein.

KANEKO's low values from his measurements on dogs seem hard to explain. The three other investigators used rabbits, and these values agree well with each other.

It cannot be regarded as certain that the blood flow through each vortex vein is the same. Secondly, the possibility cannot be overlooked that the normal distribution of the blood flow between the four vortex veins may be disturbed by the surgical handling. Thus, if the values obtained from one vortex vein are multiplied by 4 in order to obtain a value for the uveal circulation, an error may be introduced, the magnitude of which cannot be determined. There exists, however, no better method for estimating total blood flow through the uveal tract.

The changes which take place in the rate of secretion of ascorbic acid into the aqueous humour when the rate of blood flow in the eye is altered by ligation of the common carotid artery, or by other means, have been investigated (Linnér 1951 a, 1951 b, 1952). The purpose of these experiments was to discover if the rate of secretion of ascorbic acid from the plasma into the aqueous humour is limited by the rate of blood flow through the secretory part of the eye. It was found that the concentration of ascorbic acid in the aqueous humour varies when the rate of blood flow is changed. As long as the secretory capacity is not exceeded, the ratio between the concentration of ascorbic acid in the aqueous humour on the ligated, and on the non-ligated, side is constant, and independent of variations in the concentration of ascorbic acid in the plasma. When, however, the secretory capacity is exceeded, the rate of blood flow can no longer be the limiting factor, and the difference caused by carotid ligation at lower ascorbic-acid-levels disappears. These results support the theory that the rate of secretion of ascorbic acid into the aqueous humour is determined by the rate of blood flow in the secretory part of the eye.

Unilateral carotid-ligation in rabbits reduces the concentration of ascorbic acid in the aqueous humour to 83 per cent of that of the control eye. According to the author's theory this value reflects the reduction in the rate of blood flow. However, it is much smaller than the reduction of the mean blood-pressure-head, which is normally about 65 (*i.e.* 95—30) mm Hg, and which drops to 38 (*i. e.* 64—26) mm Hg 24 hours after carotid ligation. (The arterial blood-pressure decreases from about 95 to 64 mm Hg, and the intraocular pressure from about 30 to 26 mm Hg.) This means a decrease to 58 per cent of the original value (Bárány 1946, 1947). According to Bárány's measurements, the rate of blood flow through the external ear is reduced to below 65 per cent, because the heat-dissipation was found to drop to that amount.

The ocular circulation has such a special character that it cannot be unreservedly compared with that of other organs. However, the difference between the effect of carotid ligation on the mean pressure-head, and on the blood flow through the ear on the one hand, and on the other hand on the rate of secretion of ascorbic acid into the eye, is strikingly great. Therefore it seemed important to measure the rate of blood flow through

the eye by a completely independent method, to check the results obtained by the ascorbic acid method. This can be accomplished by sampling at the same time blood from symmetrically situated vortex veins in both eyes, one eye being influenced by carotid ligation, and the other not.

## Materials and Methods.

Male albino rabbits weighing about 2 kg were used. The animals were kept on a diet of hay, fresh grass, oats, and water, ad lib. The common carotid artery on one side was ligated under intravenous anaesthesia using a short-acting barbiturate (Numal 'Roche' 0.6 ml/kg body weight). 18—20 hours later the rabbits were anaesthetized in the same way and the experiments were performed. After canthotomy the upper eyelid was raised by a suture-thread and the conjunctiva was loosened with great care from the upper part of the eyeball. The superior rectus muscle was cut through, and the eyeball was fixed and turned slightly downwards by a suture-thread through the insertion of the muscle. Any extreme deviation from the normal position was carefully avoided in order to prevent stasis, and a redistribution of the blood-flow, in the four vortex veins. The vortex vein running along the nasal border of the superior rectus muscle could now be exposed where it emerges from the sclera. The operation was carried out by the same operator in an exactly similar way on both eyes, and with great care to prevent bleeding. The rabbit was now given 0.5 ml 5 per cent heparin 'Vitrum' intravenously. A centrifugal back-flow of the blood had to be avoided, so the operator and his assistant occluded the chosen vortex vein in each eye by a ligature at exactly the same moment.

In order to prevent stasis, both veins were opened only a few seconds later by a longitudinal incision of 2—3 mm at the point where they leave the sclera. Because of the low blood-pressure in the vein, there was some danger that the blood flow from the opening might stop. In order to avoid this, a longitudinal incision was preferred to the more usual method of opening the vein. Filter-papers were weighed in advance in covered weighing-bottles. The blood from the veins was collected on filter-papers at the same time on both sides for a period of 45—60 seconds. The filter-papers were slowly revolved during the process to prevent oversaturation, and were immediately placed in weighing-bottles, covered, and reweighed. The amount of blood was the difference between the two weighings. The same procedure was carried out five times in immediate succession on each rabbit, and the mean value was used.

While the blood is being absorbed by the filter-paper a certain amount of evaporation takes place. This was measured by spreading heparinized rabbit-blood along the edge of a filter-paper placed on an uncovered balance-pan. In six experiments the loss of weight in

one minute averaged 0.95 per cent of the original weight of blood, which varied between 0.526 and 0.374 g.

Standard statistical methods have been used in accordance with the formulae given by BONNIER-TEDIN (1940). The standard error of a single determination was calculated by means of analysis of variance as 'variance within classes'.

## Results and Discussion.

Table 1 shows the amount of blood which escapes from one single vortex vein in one minute on the non-ligated, and on the ligated, sides, and finally the ratio between the values from the two sides. The variations between the five blood-samples taken in immediate succession from each eye have been used in order to measure the error of the method. This includes the errors connected with the taking of blood-samples from a single vein, and also the variations in the rate of blood flow through the eye which can take place from minute to minute. The error of the method is so small that its contribution to the variations in results from rabbit to rabbit is insignificant.

**Table 1.**

| | Eye | | Ratio: Ligated / Non-ligated |
|---|---|---|---|
| | Non-ligated side | Ligated side | |
| The amount of blood from one vortex vein in g/minute. For each eye the average of 5 blood samples taken in immediate succession is used. 20 rabbits. | m = 0.448<br>s = 0.102<br>e = ± 0.023<br>n = 20 | m = 0.363<br>s = 0.117<br>e = ± 0.026<br>n = 20 | m = 0.814<br>s = 0.173<br>e = ± 0.039<br>n = 20 |
| Standard error of a single determination. Derived from 5 blood-samples obtained in immediate succession from each eye. 20 rabbits. | s = 0.044<br>n = 100 | s = 0.052<br>n = 100 | s = 0.140<br>n = 100 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of determinations.

The value obtained from the eye on the non-ligated side is higher than that given by the authors referred to above. One cause may be the fact that modern heparin is a more efficient anticoagulant than those available to the earlier investigators.

Another cause contributing to the lower values of the earlier investigations may lie in the anaesthesia. SONDERMANN in his experiments used urethane, and MEESMANN used chloral hydrate. As is well known, they both can cause a considerable fall in blood-pressure. FISCHER does not report his method of anaesthesia.

Eyes taken from rabbits of the breed and size used in these experiments weigh 2.5 g (arithmetic mean of ten enucleated eyes from five rabbits).

The arithmetic mean of the ratio between the amount of blood on the ligated, and on the non-ligated, side is $0.81 \pm 0.039$. This agrees very well with the concentration of ascorbic acid in the aqueous humour, which, under exactly comparable conditions, dropped to $0.83 \pm 0.01$ of that of the control eye. However, the similarity may be partly fortuitous, and not only for statistical reasons. The pressure-flow diagram can be different in various parts of the uveal tract, causing an unequal change in the rate of blood flow when the blood-pressure rises or falls. The change obtained for the flow through the whole uveal tract, as found in the vortex veins, is not necessarily representative of conditions in the ciliary processes. Moreover, carotid ligation may affect the rate of flow of aqueous humour. If this is so, the ascorbic acid concentration alone does not correctly reflect the changes in the rate of secretion. Present evidence indicates that the error introduced by this factor is small. This matter will be discussed more fully in another connection.

In spite of the qualifications mentioned above, the agreement between the change in rate of blood flow from the vortex vein, and the change in concentration of ascorbic acid in the aqueous humour, seems to justify the use of ascorbic acid as a clearance-substance for measuring the rate of blood flow through the ciliary processes.

The results of these experiments show a remarkably small change (19 per cent) in the rate of blood flow, in view of the considerable reduction (about 42 per cent) in pressure-head caused by ligation of the carotid.

FOLKOW (1949), in his experiments on cats, has shown that if a hind limb is totally denervated the arterioles dilate when the blood-pressure is reduced. In his opinion, mechanical factors provided by changes in blood-pressure play a rôle in the dilatation of the vessels. Under more physiological conditions, with intact innervation, arterioles with a diameter of about 50 mi-

crons have been investigated, in the pia mater (Forbes, Nason and Wortman, 1937, Fog 1937), and in the retina (Porsaa 1941).

Porsaa measured the diameter of the arterioles in the retina of cats, and the changes which follow variations in the arterial blood-pressure. He found, in conformity with results from the vessels of the pia mater, that the arterioles dilated when the arterial blood-pressure was lowered. The result was the same, no matter how the blood-pressure was reduced. The vessels he was able to measure had a diameter of about 50 microns. The main resistance, of course, lies in still smaller arterioles. Provided that the measured changes in diameter reflect at least approximately the changes in those lumina which determine the resistance, Porsaa's values can be used for estimating roughly the changes in the vascular resistance in the retina, when the blood-pressure is reduced, by a simple application of Poisseuille's law.

**Table 2.**

*The effect of mean pressure-head on the diameter of the retinal arterioles in cats.*

Blood-pressure and intraocular pressure in mm Hg. The diameter of the arterioles recalculated to microns from Porsaa's data.

| | $P_a$ | $P_i$ | $P_a—P_i$ | D | R |
|---|---|---|---|---|---|
| Normal arterial blood-pressure. | 126 | 20 | 106 | 46 | 1 |
| Decreased arterial blood-pressure. | 72 | 16 | 56 | 54 | 0.53 |

Symbols: $P_a$ = arterial mean-blood-pressure
$P_i$ = intraocular pressure
$P_a—P_i$ = mean pressure head
D = arterial diameter
R = vascular resistance, relative value

Table 2 shows the changes in vascular resistance calculated from Porsaa's values in comparison with the changes in mean pressure-head. Porsaa gives the value of the arterial blood-pressure ($P_a$) but he gives no information concerning the intraocular pressure ($P_i$). The mean pressure-head is $P_a - P_i$. Magitot and Baillart (1919) give a value of 15–25 mm Hg for the intraocular pressure in cats. A value of 20 mm Hg is used here. $P_i$ is supposed to drop 4 mm Hg, *i.e.* as much as Bárány has found

in rabbits after ligation of the carotid artery. This reduction of 4 mm seems reasonable because the fall in the pressure is about the same in the experiments of Bárány and Porsaa.

When the mean pressure-head ($P_a$—$P_i$) is lowered to 53 per cent, the vascular resistance too falls to 53 per cent. Thus, the rate of blood flow through the retina would not seem to change.

A comparison between the uveal tract and the retina shows that vascular resistance in both is reduced when the blood pressure falls. The haemodynamics of the eye are extremely complex; and the figures refer to different kinds of animals, and different, though comparable, physiological phenomena. Thus, the calculations here are impaired by a great uncertainty and cannot be used for very far-reaching conclusions. It seems justifiable, however, to draw one conclusion: the retina and the uveal tract react in the same way to a reduction of blood-pressure, namely by a lowering of the vascular resistance.

## Summary.

The rate of blood flow through the uveal tract of the eye can be determined by measuring the amount of blood escaping from an opened vortex vein. In this way the uveal blood flow in rabbits was measured at the same time on both eyes 18—20 hours after unilateral ligation of the common carotid artery. On the ligated side the rate of blood flow amounted to 81 $\pm$ 4 per cent of that on the non-ligated side. This difference corresponds with the concentration of ascorbic acid in the aqueous humour, which is reduced to 83 $\pm$ 1 per cent after the ligation of the carotid artery. This agreement supports the theory that the rate of secretion of ascorbic acid into the aqueous humour reflects the rate of blood flow through the secretory part of the eye.

This investigation was supported by a grant from the Regnell Fund of the University of Uppsala. The technical assistance of Mr. A. Ojamaa is gratefully acknowledged.

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*EXCERPTUM*

Acta Physiologica Scandinavica 1952. Vol. 26. Fasc. 1.

From the Pharmacological Institute of the University of Uppsala

# Ascorbic Acid as a Test Substance for Measuring Relative Changes in the Rate of Plasma Flow through the Ciliary Processes.

## III. The Effect of Pre-ganglionic Section of the Cervical Sympathetic in Rabbits on the Ascorbic Acid Content of the Aqueous Humour at Varying Plasma Levels.

By

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Received 1 March 1952.

The existence of sympathetic vaso-constrictor innervation of the uveal tract can be considered as proved. (For reviews see Duke-Elder 1932, Adler 1950.) However, it has so far not been possible to make quantitative estimations of the normal nervous tone. In the present paper the concentration of ascorbic acid in the aqueous humour has been used as an indicator of circulatory changes in the ciliary processes (Linnér 1951 a, 1951 b, 1952) caused by section of the cervical sympathetic.

### Materials and Methods.

Male albino rabbits weighing 1.8—2.5 kg were used. The pre-ganglionic fibres of the cervical sympathetic were divided under a short-acting barbiturate (Numal "Roche" 0.6 ml/kg body-weight). 18—20 hours later aqueous samples were collected from the two eyes, one immediately after the other, and a blood sample was obtained by cardiac puncture. The ascorbic acid was then titrated with dichlorophenol indophenol. Before the samples were taken the success of the operation was verified by observing the size of the pupils, and the difference in temperature between the two ears when the animals were exposed to cold. The technique, which was also used in an earlier investigation, has been described in detail in a previous paper (Linnér 1952).

The rabbits in one group were given Dibenamine intravenously. The Dibenamine solution was prepared in the following manner. 150 mg was suspended in 12 ml of a 0.9 per cent NaCl solution and 1 ml 1 N. HCl. By cautious heating a clear solution was obtained. The acidity was then adjusted to pH 1.5 by means of 1 N. NaOH and the volume was made up to 15 ml with the saline. A freshly prepared solution was injected intravenously and the vein was afterwards washed with saline.

## Results.

The concentration of ascorbic acid can be regarded as equal in the two eyes, under normal conditions (Linnér 1952).

**Table 1.**

*The effect of pre-ganglionic section of the cervical sympathetic on the ascorbic acid content of the aqueous humour, at ascorbic acid levels below saturation level.*

The sympathetic was cut on one side 18—20 hours before removal of the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

| | Ascorbic acid in mg% | | | Ratio: Cut / Non-cut |
|---|---|---|---|---|
| | Aqueous humour | | Plasma | |
| | Non-cut side | Cut side | | |
| The whole series of 42 rabbits | m = 23.46<br>s = 5.22<br>e = ±0.81<br>n = 42 | m = 26.02<br>s = 5.93<br>e = ±0.91<br>n = 42 | m = 1.21<br>s = 0.53<br>e = ±0.08<br>n = 34 | m = 1.11<br>s = 0.07<br>e = ±0.01<br>n = 42 |
| Group of rabbits with a low ascorbic acid level (10—21 mg% in the control eye) | m = 17.88<br>s = 2.93<br>e = ±0.81<br>n = 13 | m = 20.18<br>s = 3.98<br>e = ±1.10<br>n = 13 | m = 1.11<br>s = 0.26<br>e = ±0.08<br>n = 11 | m = 1.12<br>s = 0.09<br>e = ±0.02<br>n = 13 |
| Group of rabbits with a high ascorbic acid level (26—36 mg% in the control eye) | m = 30.67<br>s = 3.26<br>e = ±1.03<br>n = 10 | m = 33.51<br>s = 4.30<br>e = ±1.36<br>n = 10 | m = 1.25<br>s = 0.21<br>e = ±0.07<br>n = 8 | m = 1.09<br>s = 0.07<br>e = ±0.02<br>n = 10 |
| Group of rabbits with the stems of the kidney tied, and given PACA | m = 23.37<br>s = 5.77<br>e = ±1.15<br>n = 25 | m = 25.80<br>s = 6.59<br>e = ±1.32<br>n = 25 | m = 1.11<br>s = 0.20<br>e = ±0.04<br>n = 25 | m = 1.10<br>s = 0.08<br>e = ±0.02<br>n = 25 |

Symbols: m = arithmetic mean  e = standard error of the mean
s = standard deviation  n = number of experiments.

Table 1 shows the ratio between the ascorbic acid content in the aqueous humour of the eye with the cut cervical sympathetic, and that in the control eye. Over the whole series of 42 rabbits the mean ratio is $1.11 \pm 0.01$. The rabbits with low

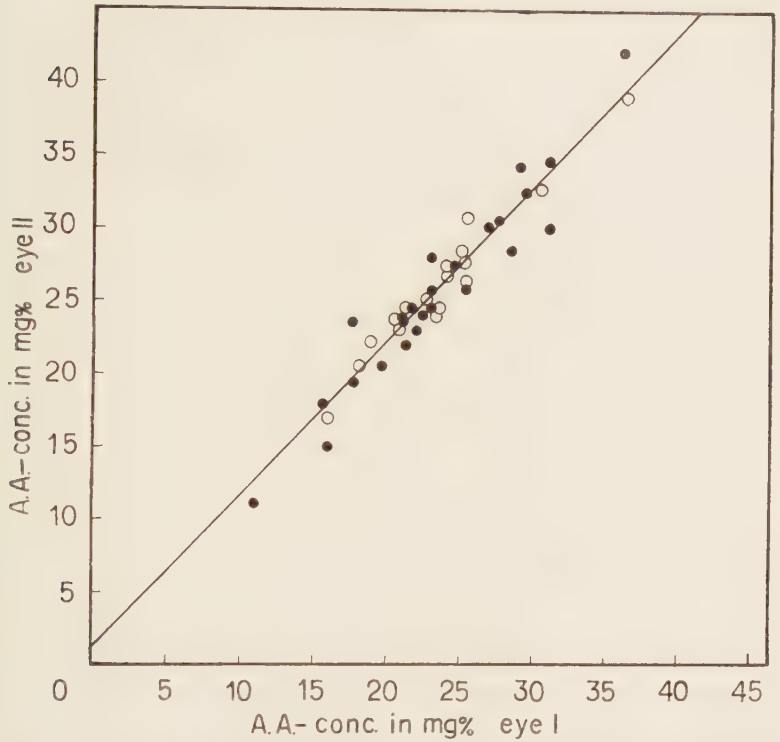


Figure 1. The effect of pre-ganglionic section of the cervical sympathetic on the ascorbic acid content of the aqueous humour, at plasma ascorbic acid levels below saturation level. The sympathetic was cut 18—20 hours before removal of the aqueous humour. Eye I: control eye. Eye II: eye with cut sympathetic. Concentrations given in mg ascorbic acid per 100 ml. *Symbols:* Open circles: rabbits with cut sympathetic only. Black dots: rabbits with cut sympathetic, the stems of the kidney tied, and given PACA.

ascorbic acid levels (10—21 mg per cent in the control eye) have a corresponding ratio of 1.12, and the rabbits with spontaneously high ascorbic acid levels (26—36 mg per cent in the control eye) have a ratio of 1.09. The difference between them is not statistically significant ($0.1 < P < 0.2$).

Figure 1 shows a scatter diagram of the results, with the control eye on the abscissa. The equation was calculated to:

$$Y = 0.88 + 1.07\ x.$$

A more far-reaching statistical analysis has been avoided for the same reasons as in the earlier paper (Linnér 1952), where one common carotid artery was ligated instead of cutting the cervical sympathetic. By analogy with the results obtained in

**Table 2.**

*The effect of pre-ganglionic section of the cervical sympathetic on the ascorbic acid content of the aqueous humour at ascorbic acid levels above saturation level.*

The sympathetic was cut on one side 18—20 hours before removal of the aqueous humour. The saturation-level was exceeded by means of intravenous infusion of an ascorbic acid solution over a period of 6 hours; plasma I was removed after 3 hours and plasma II at the same time as the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

| Ascorbic acid in mg% | | | | Ratio: |
|---|---|---|---|---|
| Aqueous humour | | Plasma I | Plasma II | Cut / Non-cut |
| Non-cut side | Cut side | | | |
| m = 49.80 | m = 50.06 | m = 9.10 | m = 11.99 | m = 1.01 |
| s = 5.60 | s = 4.85 | s = 3.13 | s = 3.75 | s = 0.05 |
| e = ±1.32 | e = ±1.14 | e = ±0.76 | e = ±0.88 | e = ±0.01 |
| n = 18 | n = 18 | n = 18 | n = 18 | n = 18 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments.

this investigation it seems reasonable to say that the scatter diagram is best represented by a straight regression-line.

In one group of rabbits the distribution of para-acetyl-amino-hippuric acid (PACA) between plasma and aqueous humour was determined at the same time as the ascorbic acid. 18—20 hours before puncture of the eyes the stems of the kidneys were tied and the rabbits given PACA. The ascorbic acid ratio between the two eyes is 1.10 in this group, compared with 1.11 in the whole series. The difference between them is not statistically significant.

As in the experiments on rabbits where one carotid artery was ligated (Linnér 1952), the maximal rate of transfer of ascorbic acid across the blood-aqueous barrier was exceeded for 6 hours by means of an intravenous infusion of a solution of ascorbic acid. The plasma ascorbic acid level was higher than 3 mg per cent. As shown in table 2 there is no difference between the concentration of ascorbic acid in the eye with the cut sympathetic, and in the control eye with intact innervation.

It is not certain that the ocular effects of the sympathetic innervation are limited to smooth muscle tone. The adrenergic nerves to the ciliary processes might have a metabolic function in addition to their vasoconstrictor action. The investigation by

**Table 3.**

*The effect of Dibenamine and pre-ganglionic section of the cervical sympathetic on the ascorbic acid content of the aqueous humour.*

The sympathetic was cut on one side and Dibenamine (10 or 15 mg/kg body-weight) was given intravenously 18—20 hours before removal of the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

| Ascorbic acid in mg% | | | |
|---|---|---|---|
| Aqueous humour | | Plasma | Ratio: Cut / Non-cut |
| Non-cut side | Cut side | | |
| m = 24.09 | m = 24.63 | m = 1.10 | m = 1.02 |
| s = 4.50 | s = 5.25 | s = 0.20 | s = 0.06 |
| e = ±0.94 | e = ±1.09 | e = ±0.04 | e = ±0.01 |
| n = 23 | n = 23 | n = 23 | n = 23 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments.

Friedenwald and Buschke (1941) speaks in favour of a rôle played by adrenalin in the secretion of the aqueous humour. The origin of the adrenalin is, however, unknown, but if it comes from the adrenergic nerve endings its concentration might be influenced by cutting the cervical sympathetic. In order to separate these different effects an adrenergic blocking agent, Dibenamine, was used. According to Nickerson (1949) the active adrenergic blocking agents, such as Dibenamine, show a high degree of specific activity. "In contrast to its effectiveness in blocking adrenergic excitatory responses, Dibenamine appears to be ineffective against inhibitory, metabolic and central nervous stimulant actions of sympathomimetic agents." The following experiments were performed in order to compare this activity of Dibenamine with sympathectomy. The cervical sympathetic was cut on one side and Dibenamine was injected intravenously. 18—20 hours later the aqueous humour was sampled. Table 3 shows that there is no difference between the ascorbic acid concentration of the aqueous humour in the eye influenced by Dibenamine and cervical sympathectomy, and in the other eye influenced only by Dibenamine.

11 rabbits were given 10 mg/kg body-weight Dibenamine, and in this series the ratio was 1.04. The other 12 rabbits were given 15 mg/kg and in this series the ratio was 1.00. The difference is not statistically significant ($0.1 < P < 0.2$), which suggests an almost complete blocking-effect.

**Table 4.**

*The effect of pupillary size on the ascorbic acid content of the aqueous humour.*

One-sided illumination 5 hours before removal of the aqueous humour. Eye I was kept in shadow and eye II was illuminated. All concentrations in mg ascorbic acid per 100 ml.

| Ascorbic acid in mg% | | |
|---|---|---|
| Aqueous humour | | Ratio: |
| I | II | II/I |
| m = 18.96 | m = 19.25 | m = 1.02 |
| s = 4.50 | s = 4.41 | s = 0.05 |
| e = ±1.01 | e = ±0.99 | e = ±0.01 |
| n = 20 | n = 20 | n = 20 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments.

In spite of these findings it is still not clear whether the influence of the cervical sympathetic is limited to the excitatory effects, which are blocked by Dibenamine; a sympathetic vasodilatator effect and a metabolic effect cannot be excluded, but, if they exist, they must cancel each other out almost exactly in order for the ratio to be 1.02, as was found. There is no experimental evidence to support such a possibility. Therefore the effect of the cervical sympathetic on the ciliary processes is probably limited to vasoconstriction.

Sympathectomy caused a difference in the size of the pupil on the cut side and in the control eye. In order to investigate if the pupillary size alone had any influence on the ascorbic acid content, the following experiments were performed. In a series of rabbits one eye was exposed for 5 hours to the light of a 100-watt lamp at a distance of 75 cm, and the other eye was kept in shadow. The difference in pupillary size was not maximal, but was of the same order as that observed in the main series following sympathectomy. The aqueous samples were collected first from the eye in shadow and immediately afterwards from the illuminated eye. Table 4 shows that there is no difference in the concentration of ascorbic acid in the aqueous humour between the two eyes. These findings did not agree with the results of earlier authors. Bonsignore (1935), using the light of a 300-watt lamp at a distance of 50 cm, and Utsumi (1937), giving no information concerning the in-

tensity of light, found an increase in the ascorbic acid concentration of the aqueous humour on exposure of the rabbits to light. The difference in intensity of light might explain the discrepancy in the results.

A full discussion will follow in a later paper.

## Summary.

Using male albino rabbits, the effect of unilateral section of the cervical sympathetic on the concentration of ascorbic acid in the aqueous humour was investigated at varying plasma-levels of ascorbic acid. Over a wide range below saturation level the ratio between the concentrations in the aqueous humour on the cut and the non-cut side was constantly $1.11 \pm 0.01$. No difference was found between the two eyes when the plasma concentration was above saturation level, or after administration of Dibenamine. A moderate change in the size of the pupil alone had no influence on the ascorbic acid concentration in the aqueous humour.

This investigation was supported by a grant from the Regnell Fund of the Faculty of Medicine in the University of Uppsala. Dr. Becker is a member of the staff of the Wilmer Ophthalmological Institute of the Johns Hopkins Hospital and University of Baltimore, Maryland, U. S. A. The Dibenamine was kindly supplied by Mr. Askelöf of A.B. Pharmacia, Uppsala. The technical assistance of Mr. A. Ojamaa is gratefully acknowledged.

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*EXCERPTUM*

**Acta Physiologica Scandinavica 1952. Vol. 26. Fasc. 1.**

From the Pharmacological Institute of the University of Uppsala.

# Ascorbic Acid as a Test Substance for Measuring Relative Changes in the Rate of Plasma Flow through the Ciliary Processes.

## IV. The Effect of Carotid Ligation and Cervical Sympathectomy in Guinea-pigs on the Ascorbic Acid Content of the Aqueous Humour at Varying Plasma Levels.

By

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Received 12 March 1952.

In the present paper the concentration of ascorbic acid in the aqueous humour of guinea-pigs has been used as an indicator of circulatory changes in the ciliary processes at plasma-concentrations varying over a wide range. The same principles were followed as in the investigations in rabbits (Linnér 1951 a, 1951 b, 1952 a, 1952 c). The blood flow through one eye was changed by means of unilateral carotid ligation, or by pre-ganglionic section of the cervical sympathetic and a study made of the ratio between the concentration of ascorbic acid in the aqueous humour of the two eyes.

### Materials and Methods.

Male guinea-pigs weighing 400—900 g were used. They were kept on a diet of hay, oats, turnips, and water, *ad lib.* The operations on the carotid artery, or the cervical sympathetic, and the sampling of the aqueous humour, were carried out under ether anaesthesia. The guinea-pigs in one group were given a subcutaneous infusion, over a period of 6 hours, of a freshly-made 2.2 per cent solution of ascorbic acid with pH adjusted to 5.5—6.5 by means of NaOH. The rate of infusion was 1 ml per hour. In order to get a higher ascorbic acid-level some animals

were given ascorbic acid subcutaneously 18–20 hours before sampling the aqueous humour. The technique, which was also used in earlier investigations has been described in detail in previous papers (Linnér 1952 a, 1952 c). Standard statistical methods were used according to the formulas given in Bonnier-Tedin (1940). The standard error of a single determination was calculated by means of the difference between two determinations on every sample (Dahlberg 1940).

## Results.

Table 1 shows the standard error of a single determination.

**Table 1.**

*The standard error of a single determination calculated by means of the difference between two determinations on every aqueous- or plasma-sample.*

Double determinations have been made on each of 40 samples of aqueous humour in guinea-pigs saturated with ascorbic acid and 20 samples of plasma from 20 different guinea-pigs in each group at different saturation levels.

| Ascorbic acid in mg% | | |
|---|---|---|
| | Plasma | |
| Aqueous humour | Low concentration | High concentration |
| m = 29.77<br>s = 0.63<br>n = 40 | m = 0.51<br>s = 0.02<br>n = 20 | m = 14.73<br>s = 0.28<br>n = 20 |

Symbols: m = arithmetic mean
s = standard error of a single determination
n = number of samples

Table 2 shows the ratio between the ascorbic acid content in the aqueous humour of the eye influenced by carotid ligation, and that of the control eye. In the whole series of 26 guinea-pigs the mean ratio is $0.88 \pm 0.02$. The guinea-pigs with low ascorbic acid levels (1—7 mg per cent in the control eye) have a corresponding ratio of 0.86, and the guinea-pigs with high ascorbic acid levels (11—15 mg per cent in the control eye) have a ratio of 0.91. The difference between them is not statistically significant ($0.3 < P < 0.4$).

Figure 1 shows a scatter diagram of the results, with the control eye as the abscissa. The equation of the regression-line was calculated to:

$$Y = -0.25 + 0.92\,x$$

**Table 2.**

*The effect of ligation of the common carotid artery on the ascorbic acid content of the aqueous humour at plasma ascorbic acid levels below the saturation level.*

The carotid artery was ligated 18—20 hours before removal of the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

| | Ascorbic acid in mg% | | | Ratio: Ligated / Non-ligated |
|---|---|---|---|---|
| | Aqueous humour | | Plasma | |
| | Non-ligated side | Ligated side | | |
| The whole series of 26 guinea-pigs | m = 8.51<br>s = 3.95<br>e = 0.78<br>n = 26 | m = 7.60<br>s = 3.72<br>e = 0.73<br>n = 26 | m = 0.52<br>s = 0.28<br>e = 0.05<br>n = 26 | m = 0.88<br>s = 0.09<br>e = 0.02<br>n = 26 |
| Group of guinea-pigs with a low ascorbic acid level (1—7 mg% in the control eye) | m = 3.83<br>s = 1.73<br>e = 0.58<br>n = 9 | m = 3.38<br>s = 1.62<br>e = 0.54<br>n = 9 | m = 0.38<br>s = 0.08<br>e = 0.03<br>n = 9 | m = 0.86<br>s = 0.11<br>e = 0.04<br>n = 9 |
| Group of guinea-pigs with a high ascorbic acid level (11—15 mg% in the control eye) | m = 12.61<br>s = 0.93<br>e = 0.31<br>n = 9 | m = 11.50<br>s = 1.43<br>e = 0.48<br>n = 9 | m = 0.61<br>s = 0.25<br>e = 0.08<br>n = 9 | m = 0.91<br>s = 0.08<br>e = 0.03<br>n = 9 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

Table 3 shows the ratio between the ascorbic acid content in the aqueous humour of the eye with a cut cervical sympathetic and that in the control eye. In the whole series of 36 guinea-pigs the mean ratio is $1.23 \pm 0.03$. The guinea-pigs with low ascorbic acid levels (2—8 mg per cent in the control eye) have a corresponding ratio of 1.27, and the guinea-pigs with high ascorbic acid levels have a ratio of 1.20. The difference between them is not statistically significant ($0.2 < P < 0.3$).

Figure 2 shows a scatter diagram of the results, with the control eye as the abscissa. The equation of the regression-line was calculated to:

$$Y = 0.61 + 1.16\ x$$

A more far-reaching statistical analysis has not been made for the same reasons as in the earlier paper (Linnér 1952 a). As in the experiments on rabbits, it seems reasonable to say that the two scatter diagrams in figures 1 and 2 are best represented by a straight regression-line.

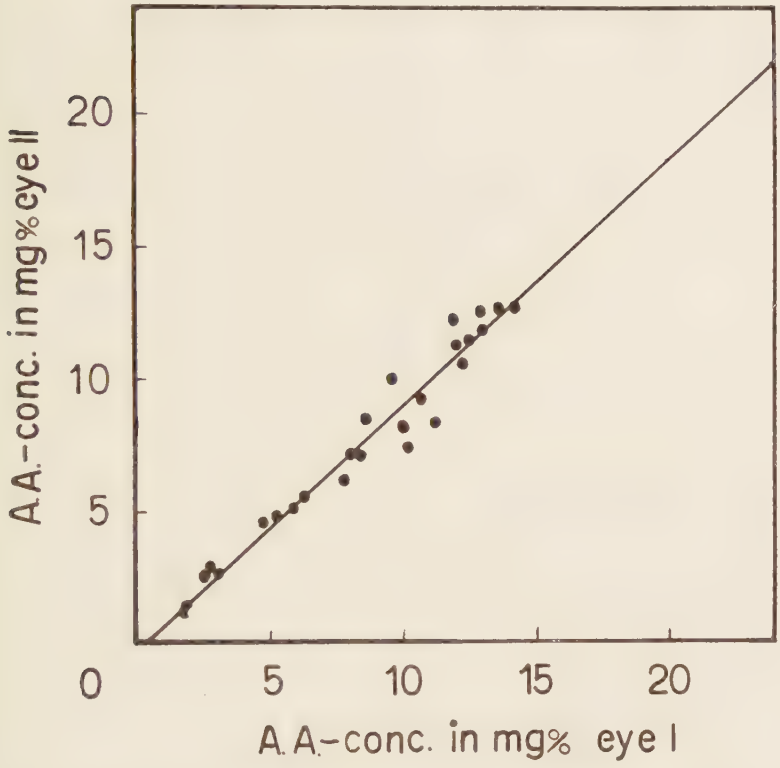


Fig. 1. The effect of ligation of the common carotid artery on the ascorbic acid content of the aqueous humour, at ascorbic acid levels below the saturation level. The carotid artery was ligated 18—20 hours before removal of the aqueous humour. Eye I: control eye. Eye II: eye influenced by carotid ligation. The concentration in mg ascorbic acid per 100 ml.

Data are also available for another acid, para-amino-hippuric acid (PAHA) which is transferred across the blood-aqueous barrier at a slow rate. This probably means that the rate of blood flow is not the limiting factor for the rate of transfer of PAHA through the blood-aqueous barrier. 37 guinea-pigs with the cervical sympathetic cut on one side were given a subcutaneous infusion of PAHA over a period of 6 hours. The ratio between the two eyes was $1.12 \pm 0.07$ and calculated as the median 1.04 (Bárány 1952). This value is probably greater than 1 even if the difference from 1 is not statistically quite significant. At a decrease in the rate of flow of aqueous humour the concentration of PAHA in the aqueous humour would increase if the transfer

**Table 3.**

*The effect of pre-ganglionic section of the cervical sympathetic on the ascorbic acid content of the aqueous humour at plasma ascorbic acid levels below the saturation level.*

The cervical sympathetic was cut 18—20 hours before removal of the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

| | Ascorbic acid in mg% | | | |
|---|---|---|---|---|
| | Aqueous humour | | Plasma | Ratio: Cut / Non-cut |
| | Non-cut side | Cut side | | |
| The whole series of 36 guinea-pigs | m = 10.57<br>s = 4.92<br>e = 0.82<br>n = 36 | m = 12.83<br>s = 5.75<br>e = 0.96<br>n = 36 | m = 0.77<br>s = 0.74<br>e = 0.12<br>n = 36 | m = 1.23<br>s = 0.15<br>e = 0.03<br>n = 36 |
| Group of guinea-pigs with a low ascorbic acid level (2—8 mg% in the control eye) | m = 5.56<br>s = 1.54<br>e = 0.41<br>n = 14 | m = 6.93<br>s = 1.74<br>e = 0.46<br>n = 14 | m = 0.46<br>s = 0.16<br>e = 0.04<br>n = 14 | m = 1.27<br>s = 0.23<br>e = 0.06<br>n = 14 |
| Group of guinea-pigs with a high ascorbic acid level (13—21 mg% in the control eye) | m = 15.91<br>s = 2.58<br>e = 0.71<br>n = 13 | m = 19.06<br>s = 2.83<br>e = 0.79<br>n = 13 | m = 1.02<br>s = 1.07<br>e = 0.30<br>n = 13 | m = 1.20<br>s = 0.06<br>e = 0.02<br>n = 13 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

of PAHA across the blood-aqueous barrier were not decreased to the same extent as the rate of flow of aqueous humour.

As shown by Kinsey (1947) and Langham (1949), the saturation of the secretory part of the eye in rabbits is reached at a plasma ascorbic acid level of about 3 mg per cent. Figure 3 shows a scatter diagram with the plasma ascorbic acid concentration in guinea-pigs on the abscissa and the ascorbic acid concentration of the aqueous humour in the control eye on the ordinate. From this scatter diagram it appears that the maximal rate of transfer of ascorbic acid across the blood-aqueous barrier in guinea-pigs is reached at a plasma ascorbic acid level of about 2 mg per cent. The concentration of ascorbic acid in the aqueous humour at saturation is about 30 mg per cent against 50 mg per cent in rabbits.

As in the experiments on rabbits, the saturation level of the guinea pigs in two groups was exceeded for 6 hours. The plasma

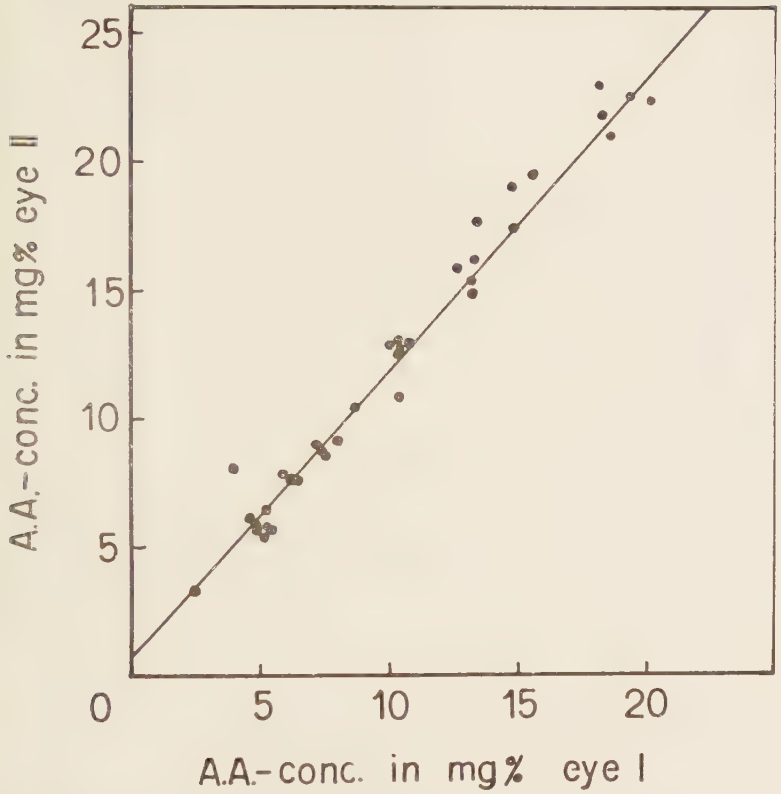


Fig. 2. The effect of pre-ganglionic section of the cervical sympathetic on the ascorbic acid content of the aqueous humour, at plasma ascorbic acid levels below saturation level. The sympathetic was cut 18—20 hours before removal of the aqueous humour. Eye I: control eye. Eye II: eye with cut sympathetic. The concentration in mg ascorbic acid per 100 ml.

ascorbic acid level was throughout higher than 2 mg per cent. As can be seen in table 4, there is no difference between the concentration of ascorbic acid in the eye influenced by carotid ligation and in the control eye. On the other hand, as shown in table 5, the ratio between the ascorbic acid content in the aqueous humour of the eye with a cut cervical sympathetic and that in the control eye is $1.08 \pm 0.008$.

As in the experiments on rabbits where one cervical sympathetic was cut some guinea-pigs were given Dibenamine in order to compare the activity of this substance alone with the effect of sympathectomy plus Dibenamine. The cervical sympathetic was cut on one side and Dibenamine was injected subcutaneously in

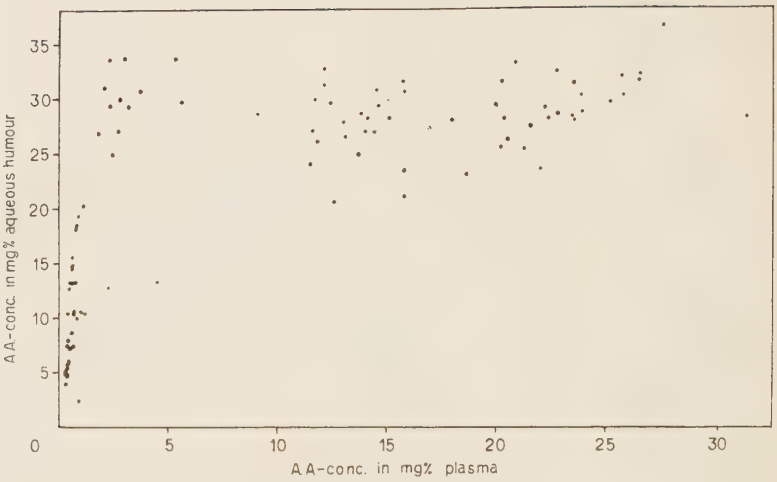


Fig. 3. The concentration of ascorbic acid in the aqueous humour at varying plasma levels showing that the maximal rate of transfer of ascorbic acid across the blood-aqueous barrier in guinea-pigs is reached at a plasma ascorbic acid level of about 2 mg per cent. Concentrations in mg ascorbic acid per 100 ml.

**Table 4.**

*The effect of ligation of the common carotid artery on the ascorbic acid content of the aqueous humour at plasma ascorbic acid levels above the saturation level.*

The carotid artery was ligated 18—20 hours before removal of the aqueous humour. The saturation level was exceeded by means of subcutaneous infusion of an ascorbic acid solution over a period of 6 hours. Plasma I was removed after 3 hours and plasma II at the same time as the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

| Ascorbic acid in mg% | | | | |
|---|---|---|---|---|
| Aqueous humour | | Plasma I | Plasma II | Ratio: Ligated / Non-ligated |
| Non-ligated side | Ligated side | | | |
| m = 29.36<br>s = 2.93<br>e = 0.61<br>n = 23 | m = 29.24<br>s = 2.97<br>e = 0.62<br>n = 23 | m = 15.36<br>s = 2.79<br>e = 0.58<br>n = 23 | m = 22.58<br>s = 2.94<br>e = 0.61<br>n = 23 | m = 1.00<br>s = 0.04<br>e = 0.009<br>n = 23 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

**Table 5.**

*The effect of pre-ganglionic section of the cervical sympathetic on the ascorbic acid content of the aqueous humour at plasma ascorbic acid levels above the saturation level.*

The cervical sympathetic was cut 18—20 hours before removal of the aqueous humour. The saturation level was exceeded by means of subcutaneous infusion of an ascorbic acid solution over a period of 6 hours. Plasma I was removed after 3 hours and Plasma II at the same time as the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

| Ascorbic acid in mg% | | | | |
|---|---|---|---|---|
| Aqueous humour | | | | Ratio: Cut / Non-cut |
| Non-cut side | Cut side | Plasma I | Plasma II | |
| m = 27.59 | m = 29.87 | m = 8.96 | m = 14.54 | m = 1.08 |
| s = 2.97 | s = 2.98 | s = 3.25 | s = 3.08 | s = 0.04 |
| e = 0.56 | e = 0.56 | e = 0.68 | e = 0.58 | e = 0.008 |
| n = 28 | n = 28 | n = 23 | n = 28 | n = 28 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

**Table 6.**

*The effect of Dibenamine and pre-ganglionic section of the cervical sympathetic on the ascorbic acid content of the aqueous humour.*

The sympathetic was cut on one side and Dibenamine was given subcutaneously 18—20 hours before removal of the aqueous humour. All concentrations in mg ascorbic acid per 100 ml.

| | Ascorbic acid in mg% | | | |
|---|---|---|---|---|
| | Aqueous humour | | Plasma | Ratio: Cut / Non-cut |
| | Non-cut side | Cut side | | |
| Guinea-pigs given 10—20 mg/kg Dibenamine | m = 14.47 | m = 15.67 | m = 0.68 | m = 1.09 |
| | s = 5.25 | s = 5.52 | s = 0.44 | s = 0.06 |
| | e = 1.46 | e = 1.53 | e = 0.12 | e = 0.02 |
| | n = 13 | n = 13 | n = 13 | n = 13 |
| Guinea-pigs given 50 mg/kg Dibenamine | m = 11.48 | m = 12.09 | m = 0.67 | m = 1.06 |
| | s = 5.95 | s = 6.00 | s = 0.42 | s = 0.06 |
| | e = 1.49 | e = 1.50 | e = 0.10 | e = 0.02 |
| | n = 16 | n = 16 | n = 16 | n = 16 |
| Guinea-pigs given 100 mg/kg Dibenamine | m = 10.63 | m = 10.78 | m = 0.50 | m = 1.02 |
| | s = 4.68 | s = 4.73 | s = 0.12 | s = 0.07 |
| | e = 1.00 | e = 1.01 | e = 0.03 | e = 0.01 |
| | n = 22 | n = 22 | n = 21 | n = 22 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

**Table 7.**

*The effect of pre-ganglionic section of the cervical sympathetic on the amount of aqueous humour which could be obtained from the anterior chamber.*

The amount of aqueous humour in mg.

| Amount of aqueous humour in mg | |
|---|---|
| Non-cut side | Cut side |
| m 43.12 | m 41.26 |
| s = 21.25 | s = 22.36 |
| e = 2.24 | e = 2.36 |
| n = 90 | n = 90 |

Symbols: m = arithmetic mean
s = standard deviation
e = standard error of the mean
n = number of experiments

6—8 different places. 18—20 hours later the aqueous humour was sampled. Table 6 shows that the ascorbic acid ratio is 1.09 when 10—20 mg/kg Dibenamine has been given. When 50 mg/kg has been given the ratio drops to 1.06, and with 100 mg/kg the ratio is 1.02. These findings, which differ from those in rabbits, will be discussed in the theoretical part.

Table 7 shows that there is no statistically significant difference in the amount of aqueous humour which was obtained from the eye with the cut cervical sympathetic and that from the control eye.

## Theoretical Part.

The following abbreviations will be used.

$T$ = rate of transfer of ascorbic acid from plasma to aqueous humour.
$T_{max}$ = maximal rate of transfer of ascorbic acid from plasma to aqueous humour.
$Fl_{pl}$ = rate of plasma flow through the ciliary processes.
$Fl_{aq}$ = rate of flow of aqueous humour.
$C_{art}$ = the concentration of ascorbic acid in the arterial plasma.
$C_{ven}$ = the concentration of ascorbic acid in the venous plasma leaving the ciliary processes.
$C_{aq}$ = the concentration of ascorbic acid in the aqueous humour.

Subscript 1 stands for the control eye.

Subscript 2 stands for the eye influenced by carotid ligation or cervical sympathectomy.
Subscript 'sat' = plasma ascorbic acid level exceeds the saturation level.

$$q = \text{the ratio } \frac{C_{aq_2}}{C_{aq_1}}$$

In all the experiments on rabbits and guinea-pigs analysed in detail here, the ascorbic acid concentration in the aqueous humour was determined. As the lens can be supposed to be of small or no importance for the ascorbic acid concentration of the aqueous humour in the species investigated here, the following equation can be used:

$$C_{aq} = \frac{T}{Fl_{aq}} \tag{1}$$

In both rabbits and guinea-pigs $\frac{C_{aq_2}}{C_{aq_1}}$ was determined after unilateral carotid ligation or sympathectomy. In each of the four experimental series the ratio

$$\frac{C_{aq_2}}{C_{aq_1}} = q \tag{2}$$

is essentially constant and statistically different from 1, when $C_{art}$ varies over a wide range below $T_{max}$. This result can be produced by changes either in T or in $Fl_{aq}$ (equation 1). Supposing that the ascorbic acid is neither stored nor used up in the epithelium, the following equations are obtained:

$$T = Fl_{pl}\,(C_{art} - C_{ven}) \tag{3}$$

$$q = \frac{Fl_{pl_2}}{Fl_{pl_1}} \times \frac{C_{art_2} - C_{ven_2}}{C_{art_1} - C_{ven_1}} \times \frac{Fl_{aq_1}}{Fl_{aq_2}} \tag{4}$$

Since the blood flowing to both eyes can be expected to have the same ascorbic acid concentration $C_{art_1} = C_{art_2}$, thus the subscripts 1 and 2 to $C_{art}$ can be omitted.

In each series $\frac{Fl_{pl_2}}{Fl_{pl_1}}$ can be regarded as essentially constant, since there is no reason why the change in $Fl_{pl}$ produced by the operations, should vary at varying ascorbic acid levels. According to Bárány (1951), $Fl_{aq}$ in guinea-pigs, unless highly scorbutic, is not influenced by variations in the ascorbic acid level. Thus in the present investigation $\frac{Fl_{aq_1}}{Fl_{aq_2}}$ is regarded as constant in each series despite varying ascorbic acid levels. From this it follows that

$$\frac{C_{art} - C_{ven_2}}{C_{art} - C_{ven_1}} = \text{constant} \tag{5}$$

in each series, regardless of variations in $C_{art}$. From the fact that the

ratio is constant at varying $C_{art}$, it follows either that $C_{ven_1}$ and $C_{ven_2}$ are so small compared with $C_{art}$ that they can be neglected, or that $C_{ven_1} = C_{ven_2}$.

Thus
$$\frac{C_{art} - C_{ven_2}}{C_{art} - C_{ven_1}} = 1 \qquad (6)$$

As shown above, $C_{ven_1} = C_{ven_2}$ at varying $C_{art}$, in spite of the fact that $Fl_{pl_1} = Fl_{pl_2}$. Thus if $C_{ven}$ is variable and not negligible it must depend only on $C_{art}$ and not on $Fl_{pl}$. If such a connection exists between $C_{art}$ and $C_{ven}$ it can only be explained by assuming that a reduction in $Fl_{pl_2}$ following carotid ligation is caused by exclusion of a certain representative part of the processus ciliares while the rest remains uninfluenced, and, further, that an increase resulting from sympathectomy correspondingly causes inclusion of a new part of the processus ciliares in the circulation, the rest remaining uninfluenced. However, a great many facts are known from experiments on the physiology of the circulation, which indicate that the effects caused by such circulatory changes as carotid ligation or sympathectomy are not limited to the above-mentioned exclusion or inclusion of a certain part of the vascular bed. Under such circumstances it is highly improbable that $C_{ven}$ is dependent only on $C_{art}$ and independent of $Fl_{pl}$. There is consequently a strong probability that $C_{ven}$ remains constant at varying $C_{art}$. The size of $C_{ven}$ cannot be determined. Thus with the methods used here, it cannot be determined if the whole amount of ascorbic acid offered to the processus ciliares ($Fl_{pl} \times C_{art}$) is transferred across the blood-aqueous barrier. A total extraction, *i. e.* $C_{ven} = 0$, presupposes that no fraction is firmly plasma-bound. Coolidge (1940), and Sargent and Golden (1950), have demonstrated experimentally that ascorbic acid is completely ultrafiltrable from plasma.

Ascorbic acid is found both in plasma and in blood corpuscles. The question is whether the exchange of ascorbic acid between the blood corpuscles and the plasma is so slow that there is not time enough for the ascorbic acid in the blood corpuscles to diffuse into the plasma while the blood is flowing through the capillaries of the processus ciliares. If this can be assumed the ascorbic acid in the blood corpuscles can be neglected; only the ascorbic acid in the plasma is of importance in the transfer to the aqueous humour. Diodrast, but not p-amino-hippuric acid, penetrates human and canine red corpuscles. In two human experiments Smith (1951) has shown that simultaneous renal clearances of these two compounds remained identical in spite of marked changes in the renal plasma flow. He concludes that the cell transport does not contribute to the Diodrast clearance. It is very probable that this result is also valid for ascorbic acid in the secretory part of the eye. But because ascorbic acid is as yet the only test substance available, no crucial experiments similar to those of Smith can be performed.

Thus there is probably nothing to prevent complete extraction, *i. e.* $C_{ven} = 0$.

According to equation 6

$$\frac{C_{art} - C_{ven_2}}{C_{art} - C_{ven_1}} \quad 1$$

This leads to a highly important simplification of equation 4:

$$q = \frac{Fl_{aq_1}}{Fl_{pl_1}} \times \frac{Fl_{pl_2}}{Fl_{aq_2}} \tag{7}$$

so long as the saturation level is not exceeded. Above this saturation level $Fl_{pl}$ can no longer be a limiting factor; thus we have

$$q_{sat} = \frac{Fl_{aq_1}}{T_{max_1}} \times \frac{T_{max_2}}{Fl_{aq_2}} \tag{8}$$

The various theoretical alternatives will now be analysed and compared with the experimental values of the ratio q.

$\frac{Fl_{aq_1}}{Fl_{pl_1}}$ refers to the control eye. Sympathectomy, whereby the depressor nerve was damaged by the technique used in these experiments, and carotid ligation, cause a slight increase in the blood pressure and some peripheral vasoconstriction. Because of this the control eye cannot be regarded as completely uninfluenced by the operation. However, since this influence is the same in both eyes, it is of no importance for q.

Thus it is sufficient to discuss $\frac{Fl_{pl_2}}{Fl_{aq_2}}$.

There are three alternatives below saturation level:

1) $Fl_{pl_2}$ and $Fl_{aq_2}$ remain unchanged, or are changed equally: q is not affected.
2) $Fl_{pl_2}$ decreases more, or increases less, than $Fl_{aq_2}$: $q < 1$.
3) $Fl_{pl_2}$ increases more, or decreases less, than $Fl_{aq_2}$: $q > 1$.

In analogy with the alternatives below the saturation level, $q_{sat}$ is determined by $\frac{T_{max_2}}{Fl_{aq_2}}$ by means of equation 8. The corresponding alternatives are:

4) $T_{max_2}$ and $Fl_{aq_2}$ remain unchanged, or are changed equally: $q_{sat}$ is not affected.
5) $T_{max_2}$ decreases more, or increases less, than $Fl_{aq_2}$ $q_{sat}$: $< 1$.
6) $T_{max_2}$ decreases less, or increases more, than $Fl_{aq_2}$ $q_{sat}$: $> 1$.

Firstly the experimental data obtained through carotid ligation will be analysed. The alternatives based on an increase of $F_{pl_2}$, $Fl_{aq_2}$ and $T_{max_2}$, will not be considered because carotid

ligation can be supposed either to have no effect, or to cause a reduction as compared with the control eye.

Below saturation level the ratio q was found to be for rabbits $0.83 \pm 0.01$ and for guinea-pigs $0.88 \pm 0.02$. This agrees with alternative 2, *i. e.* $Fl_{pl_2}$ has decreased more than $Fl_{aq_2}$. Above saturation level the ratio $q_{sat}$ was $1.01 \pm 0.008$ for rabbits and $1.00 \pm 0.009$ for guinea-pigs. This agrees with alternative 4, *i. e.* $T_{max_2}$ and $Fl_{aq_2}$ remain unchanged, or are changed equally.

From the analysis it can be concluded that carotid ligation causes a decrease in $Fl_{pl_2}$. Furthermore, it is possible but not certain that $T_{max_2}$ and $Fl_{aq_2}$ are decreased. If such a reduction takes place, it must be smaller than the reduction in $Fl_{pl_2}$, and of exactly the same degree for both $T_{max_2}$ and $Fl_{aq_2}$. This reduction in $T_{max_2}$ and $Fl_{aq_2}$ might occur, if *e. g.* lowering of the mean pressure-head causes a change in the pressure-flow diagram, whereby vessels of a certain caliber collapse completely, excluding a certain part of the ciliary processes but leaving the remainder essentially uninfluenced. By means of the ratio q alone it is only possible to measure changes in $Fl_{pl_2}$ without corresponding changes in $Fl_{aq_2}$. Because q is not influenced by such a decrease in circulation as *e. g.* an exclusion of certain groups of blood vessels it gives only a minimum value of the real change in $Fl_{pl_2}$. A simultaneous measurement in each eye separately of $C_{aq}$ and $Fl_{aq}$ would be necessary to give the real change. At present, however, there are no suitable methods available for studies of this kind in animals. In the single human eye, $Fl_{aq}$ can be estimated by means of Goldmann's fluorescein method (Goldmann 1950).

Thus, by means of the results obtained with ascorbic acid alone it is not possible to say if $Fl_{aq_2}$ is changed. There is, however, a good agreement between the value of the ratio q below saturation level in rabbits, 0.83 (Linnér 1952 a), and the ratio between the amount of blood escaping from an opened vortex vein on the ligated and on the non-ligated side, 0.81 (Linnér 1952 b). This speaks in favour of $Fl_{aq_2}$ not being changed to any great extent by carotid ligation. Furthermore the rate of disappearance of sodium is only slightly decreased by carotid ligation (no statistically significant decrease: Bárány 1947; $7 \pm 1.5$ % Davson and Matchett 1951). It is probable therefore that the effect of carotid ligation in the rabbit is of the order of 25 % reduction in plasma flow through the ciliary processes.

In the experiments with cutting of the cervical sympathetic a

difference was found between the two species. In the rabbits the ratio q was found to be $1.11 \pm 0.01$ at levels below saturation level. This agrees with alternative 3, *i. e.* $Fl_{pl_2}$ has increased more, or decreased less than $Fl_{aq_2}$.

In a group of rabbits with unilateral cervical sympathectomy which were given Dibenamine the ratio was found to be $1.02 \pm 0.01$. According to the discussion in a previous paper (LINNÉR 1952 c) this shows that the effect of the cervical sympathetic on the ciliary processes in the rabbit is probably limited to vasoconstriction.

Above saturation level the ratio q is 1.01 for rabbits. The result agrees with alternative 4, *i. e.* $T_{max_2}$ and $Fl_{aq_2}$ remain unchanged, or are changed equally. If such an alteration takes place it may be either an increase or a decrease. If an increase of $T_{max_2}$ and $Fl_{aq_2}$ takes place it must nevertheless be smaller than the increase of $Fl_{pl_2}$ in order to agree with the ratio 1.11 at levels below saturation level. An equally great change in $T_{max_2}$ and $Fl_{aq_2}$ might arise if new areas of blood vessels, which before were completely excluded from the circulation, were opened while the other areas remained uninfluenced. The situation thus is the exact counterpart of that discussed above in connection with carotid ligation.

On the other hand, the results obtained from the guinea-pigs were different. The ratio q is $1.23 \pm 0.03$ at levels below saturation. This agrees with alternative 3, *i. e.* $Fl_{pl_2}$ has increased more, or decreased less, than $Fl_{aq_2}$. Above saturation level the ratio q is $1.08 \pm 0.008$. This agrees with alternative 6, *i. e.* $T_{max_2}$ has decreased less, or increased more than $Fl_{aq_2}$. The reduction of the ratio from 1.23 to 1.08 is probably analogous to the reduction from 1.11 to 1.01 observed in the rabbits. But the ratio 1.08 above saturation level which is statistically different from 1 remains to be explained. We will return to this question in connection with the discussion of the results obtained with Dibenamine.

10—20 mg/kg body-weight of Dibenamine gave a ratio q of $1.09 \pm 0.02$; 50 mg/kg reduced q to $1.06 \pm 0.02$ and after doses as large as 100 mg/kg q was $1.02 \pm 0.01$.

The ratio obtained by means of p-amino-hippuric acid in 37 guinea-pigs was found to be 1.04 (median). This result speaks in favour of a decrease in $Fl_{aq_2}$.

The ratios obtained after sympathectomy both at levels above saturation and with Dibenamine can be explained by a decrease

in $Fl_{aq_2}$ or an increase in $T_{max_2}$ and $T_2$. There are conceivably several complicated mechanisms which could cause such a result. Here an explanation will be offered, which seems to the author to be the most probable one. There is, however, no conclusive evidence to prove its correctness.

The innervation to the ciliary processes in guinea-pigs (but not in rabbits) might include sympathetic secretory fibres. These fibres would have to be of greater importance for the transfer of water than of ascorbic acid across the blood-aqueous barrier. Furthermore, larger doses of Dibenamine would be required to block the secretory impulses than the vasoconstrictor impulses to the ciliary processes.

This explanation is based on three separate hypotheses. The existence of secretory fibres to the ciliary processes has never been proved but is compatible with a large body of clinical observations showing a rise of intraocular pressure in connection with overactivity of the sympathetic nervous system (for references see Duke-Elder 1940).

There are some clinical observations showing that Dibenamine has a lowering effect on the intraocular pressure in some types of glaucoma (Christensen and Swan 1949, Bloomfield and Haimovici 1950, Newell, Ridgeway and Zeller 1951, Clark and Duggan 1951, Muller and Leopold 1951). Assuming that there are sympathetic secretory fibres to the ciliary processes in the human eye in the same way as has been assumed for guinea-pigs and that the secretory impulses could be blocked by Dibenamine such a blocking effect might play a rôle in lowering the intraocular pressure in glaucoma.

The second hypothesis is that the secretory fibres mainly activate those secretory elements which are responsible for the bulk of the aqueous. There is no positive evidence for or against this hypothesis but the present experiments as well as those of Bárány (1951) at least show that transfer of water is not intimately connected with transfer of ascorbic acid. Whether the two are effected by separate anatomical structures or not is an open question. The third hypothesis, finally, is that the secretory fibres are more resistant to Dibenamine than vasomotor fibres. There are many examples of such differences between various functional systems in the sensitivity to autonomic blocking agents. It should be mentioned however, that Uvnäs (1948) found the ordinary dose of 10–20 mg/kg Dibenamine sufficient for complete blocking of

the sympathetic secretory impulses to the submaxillary gland of the cat. One has, of course, to be cautious in drawing conclusions from the submaxillary gland in the cat to the ciliary processes in the guinea-pig. Furthermore, in the present experiments Dibenamine was injected subcutaneously, which might be less efficient than the intravenous, intramuscular or intraperitoneal injection used by Uvnäs.

The final elucidation of these questions will depend upon simultaneous measurements of $C_{aq}$ and $Fl_{aq}$.

Summarizing the present discussion it can be said that its main point is the establishment of equation 6:

$$\frac{C_{art_2} - C_{ven_2}}{C_{art} - C_{ven_1}} = 1$$

which is based on four series of subsaturation experiments in two species. It has as a consequence that

$$q = \frac{Fl_{aq_1}}{Fl_{pl_1}} \times \frac{Fl_{pl_2}}{Fl_{aq_2}}$$

The importance of this relation is that q below saturation level is independent of variations in the plasma ascorbic acid concentration, and dependent only on $Fl_{pl}$ and $Fl_{aq}$. This means that ascorbic acid can be used to measure relative changes in the rate of blood flow through the ciliary processes at least in rabbits and guinea-pigs. A valuable check of the method has been obtained by comparison with direct measurements of the blood flow in a vortex vein. After ligation of the carotid artery in rabbits q was 0.83 (Linnér 1952 a), while the ratio between the amount of blood escaping from an opened vortex vein on the ligated and on the non-ligated side was 0.81 (Linnér 1952 b).

## Summary.

The concentration of ascorbic acid in the aqueous humour was investigated at varying plasma levels of ascorbic acid in male guinea-pigs, in one group after unilateral carotid ligation, and in another group after unilateral section of the cervical sympathetic. Over a wide range below saturation level the ratio between the concentrations in the aqueous humour of the two eyes was 0.88 $\pm$ 0.02 in the group where the carotid artery was ligated, and 1.23 $\pm$ 0.03 in the group where the cervical sympathetic was cut.

After carotid ligation no difference was found between the two eyes above saturation level. After section of the cervical sympathetic, on the other hand, the ratio between the two eyes was found to be $1.08 \pm 0.008$ above saturation level. After unilateral section of the cervical sympathetic together with subcutaneous administration of 10–20 mg/kg body-weight Dibenamine the ratio between the two eyes was found to be $1.09 \pm 0.02$, after 50 mg/kg the ratio was $1.06 \pm 0.02$ and after 100 mg/kg $1.02 \pm 0.01$. After section of the cervical sympathetic no difference was found between the amounts of aqueous humour in the two eyes.

An analysis of the present results and those previously obtained in rabbits shows that at concentrations below saturation level ascorbic acid is either completely removed from the plasma flowing through the ciliary processes or reduced in concentration down to a constant level in the effluent plasma. This fundamental fact makes possible the use of ascorbic acid as a test substance for measuring relative changes in the rate of plasma flow through the ciliary processes.

This investigation was supported by grants from the Research Fund of the Faculty of Medicine in the University of Upsala, and from the Harald and Greta Jeansson Foundation. The technical assistance of Miss K. Nordström, Miss I. Dalhagen, Mrs. L. Väli and Mrs. M. Edström is gratefully acknowledged.

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*Kungl. Boktr. Sthlm 1952*
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